

Intestinal Excretion of Endogenous Zinc in Guatemalan School Children^{1,2}

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Abstract

The intestine is the major route of excretion of endogenous zinc (Zn) and has a key role in maintaining Zn homeostasis. The principal objective of this paper is to provide an interpretative report of quantities of endogenous fecal Zn (EFZ) excreted by rural Guatemalan school children fed either normal or low phytate maize as their principal food staple. EFZ was measured by a Zn stable isotope technique. EFZ did not differ between control and low phytate maize groups. The overall EFZ ($n = 53$) was (mean \pm SD) 1.56 ± 0.69 mg Zn/d or 0.07 ± 0.03 mg Zn/kg body wt⁻¹·d⁻¹. EFZ was not correlated with the quantity of Zn absorbed. The estimated EFZ at the level of absorption that matched the physiologic requirement (EFZ_{PR}) did not differ from the above mean value. The EFZ_{PR} of 0.07 ± 0.03 mg Zn/kg body wt is twice the value currently used in the estimation of Dietary Reference Intakes. Supported by other recent childhood data, these results suggest that the current estimates of EFZ_{PR} used in the calculation of Zn requirements for children are misleadingly low. J. Nutr. 137: 1747–1749, 2007.

Introduction

Quantitative information on the excretion of endogenous zinc (Zn)⁷ via the intestine is essential for determining physiologic requirements of this notable micronutrient (1,2). Such information is remarkably limited, especially for age groups other than adults and, to some extent, young infants (3,4). This limitation is especially important, because data on physiologic requirements is a core requirement for any factorial approach to the estimation of dietary Zn requirements. At this time, a factorial approach is the only available means of estimating these requirements (5).

The objectives of this study were to measure endogenous fecal Zn (EFZ) in school-aged children while consuming their habitual high-maize diet and, as part of a study previously reported (6), to also measure EFZ in children from the same poor Guatemalan village when fed a low-phytate maize.

Methods

Study design. This was a cross-sectional study of school-aged children, residents of the poor hill village of Buena Vista in the Western Highlands

of Guatemala. The details of the study population, design, and most of the methods were published previously (6). In brief, 60 school-aged children were randomized in a convenience sample to consume either a low-phytate maize (*lpa1-1*), its isohybrid wild type, or a local control maize for a 10-wk period, during the last 2 wk of which the participants would each have a Zn stable isotope/metabolic collection study, with EFZ as the primary outcome for the component of the study reported here.

Subjects. Sixty children (29 males and 31 females) aged (mean \pm SD) of 8.9 ± 1.3 y participated in this study. Intravenous isotope administration was incomplete in 1 subject and 6 subjects reported incomplete metabolic sample collections.

Ethics. The study was approved by the Center for Studies of Sensory Impairments, Aging, and Metabolism Human Subjects Committee and by the Colorado Multiple Institutional Review Board. The consent form was written in Spanish and explained both in Spanish and Kaqchikel, the local ethnic language, with the support of resident health workers in Buena Vista. The study was initially described in information group sessions to anyone in the community who was potentially interested in having their family participate. After adequate time for consideration, interested families returned for further discussion and, if they elected to participate, to give written informed consent.

Diet. Participants and their families were free-living during the 10-wk study period. They consumed their habitual diets, the only exception being that their usual maize supply was replaced by maize provided by the investigators. Families were randomized to receive a low-phytate maize, the isohybrid wild type to this low phytate maize, or a locally grown control maize. The phytate intakes were (mean \pm SD) 1536 ± 563 mg/d for the *lpa1-1* low phytate group, 2056 ± 517 mg/d for the wild-type control, and 2253 ± 687 mg/d for the local maize control groups (6). Corresponding figures for phytate:Zn molar ratios were 18 ± 5 , 26 ± 6 , and 23 ± 5 , respectively (6).

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⁷ Abbreviations used: EFZ, endogenous fecal zinc; EFZ_{PR}, EFZ at the level of absorption that matches the physiologic requirement; TAZ, total absorbed zinc; Zn, zinc.

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Isotope preparation. Accurately weighed quantities of preparations of Zn oxide enriched with ^{67}Zn (Trace Sciences International) were dissolved in 0.5 mol/L H_2SO_4 to prepare a stock solution. The pH of the stock solution was adjusted to 6.0 with ammonium hydroxide and the stock solution was diluted with sterile isotonic sodium chloride to a Zn concentration of 1.5 mmol/L. The solution was filtered through a 0.2- μm filter. The Zn concentrations were measured by atomic absorption spectrophotometry with mass correction factor applied (7). Accurately weighed quantities were stored in sealed sterile vials and tested for pyrogens and sterility before use.

Isotope administration. After 8 wk of consumption of the low-phytate or 1 of the control maize varieties, EFZ was measured using an isotope dilution technique (8). An accurately weighed quantity (~ 0.800 mg) of ^{67}Zn (90.9% purity) was administered i.v. during the afternoon on d 1. Administration was performed with a 10-mL syringe and 3-way stopcock via a scalp vein needle inserted into a superficial forearm vein over a 5–10-min interval. The syringe was flushed twice with normal saline using the 3-way stopcock.

Fecal markers. Nonabsorbable fecal markers (methylene blue, AKA brilliant blue, 1 mg/kg body wt, Warner Jenkinson) were administered with breakfast on d 3 and d 7 to demarcate the metabolic period.

Sample collection. All fecal samples from the time of the first administration of the fecal marker on d 3 until complete passage of the marker administered on d 7 were quantitatively collected in trace-metal free plastic bags. Timed spot (20–50 mL) urine samples were collected once in the morning and once in the evening from d 3 to 7. Urine and fecal samples were kept frozen at -20°C until they were transported to University of Colorado Health Sciences Center for further processing and analyses.

Laboratory analyses. Fecal samples were homogenized using a 1:1 ratio of water and duplicate aliquots (each $\sim 10\%$ of total sample) were further processed for total and isotopic Zn analyses. Fecal samples were wet digested and dry ashed prior to reconstitution in 0.1 mol/L HCl for total Zn analyses. Total Zn in the digested samples was determined by flame atomic absorption spectrophotometry. The Zn in the reconstituted fecal samples was separated from other inorganic constituents by column chromatography. Urine samples were wet digested and dry ashed prior to removing the Zn by a chelation and extraction method (9).

The ratio for ^{67}Zn : ^{66}Zn was measured in the purified fecal and urine samples using inductively coupled plasma-mass spectrometry (ICP-MS) (9). Isotope ratios were converted to percentage enrichment (defined to be all Zn in the sample from an isotopically enriched source divided by the total amount of Zn in the sample) by an algorithm that takes into account the isotope abundances and atomic mass of both the natural and the isotopically enriched Zn contained in the samples (L. Miller, unpublished data). In general, enrichment levels in the urine and fecal samples were >50 -fold above the detection limit of the analytical method.

Data processing. EFZ was calculated by an isotope dilution technique first described by Weigand and Kirchgessner (10) including the use of urine enrichment data and was subsequently first utilized in a human study by Jackson et al. (11). This involved the following formula:

$$\text{EFZ} = \sum (\text{F} \times \text{f}) / (\text{u} \times \text{t}), \text{ where F is total fecal Zn during metabolic period (milligrams),}$$

f is the corresponding fecal percent enrichment (%E) ^{67}Zn , u is mean urine %E ^{67}Zn during metabolic period, and t is time of metabolic period (4 d).

The quantity of endogenous Zn excreted in the feces when the quantity of Zn absorbed matches physiologic requirements (termed EFZ_{PR}) has been determined by the Dietary Reference Intakes Committee of the Food and Nutrition Board, National Institute of Medicine (5).

Data analysis. Data were analyzed using GraphPad Prism version 4.00 for Windows (GraphPad Software, www.graphpad.com). Group data are reported as means \pm SD and were compared by ANOVA using an α of 0.05. Pearson's correlation was used to determine the degree and

significance of association between EFZ and total absorbed Zn (TAZ). The TAZ data were reported previously (6).

Results

Fifty-three of the 60 children (8.3 ± 1.3 y) completed the study. Anthropometric details and the quantity of Zn ingested and absorbed were reported previously (6). Total fecal Zn, intestinal excretion of EFZ, and EFZ per kilogram body wt are summarized in Table 1. The maize randomization groups did not differ in total fecal Zn, EFZ, or EFZ per kilogram. EFZ and TAZ were not correlated and the EFZ_{PR} was the same as the measured mean EFZ value.

Discussion

Typically, EFZ varies directly with TAZ (1,2). The lack of a positive correlation between EFZ and TAZ was, therefore, an unanticipated result of this study. This can be attributed to relatively high intestinal losses of endogenous Zn at lower levels of TAZ, the explanation for which is uncertain.

Because EFZ is not a constant but typically varies with TAZ, caution is required in comparing EFZ for different populations or for different pathophysiologic circumstances. The value of EFZ of special significance is EFZ_{PR} (1,5). Because, in this case, there was a zero slope for EFZ vs. TAZ, this value was the same as the mean measured value of EFZ. This does not apply, however, to other available data sets (1,5).

In evaluating these results, there are only very limited data with which comparison can be made. Two other data sets are available from less-developed countries; in Malawi, for children aged 96 m and weighing 22.1 kg, EFZ_{PR} was 1.45 mg Zn/d or $65 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (12,13). In rural southeast China, children aged 23 m and weighing 10.9 kg (14) had a calculated EFZ_{PR} of 0.8 mg Zn/d or $73 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, i.e. quite close to the current results. There are no corresponding data from countries in which children of this age are considered to be well nourished. The Food and Nutrition Board, Institute of Medicine, elected to estimate EFZ_{PR} for children on a body wt basis. Relying on extrapolation from adult data, a value of $34 \mu\text{g Zn} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ was assigned for every childhood age group (5). On this basis, the EFZ_{PR} was high for each of these 3 studies of children in less-developed countries.

Zn absorption is a saturable process and age-related differences in the parameters of saturation response analysis of TAZ vs. ingested Zn are closely related to the length of the small intestine (15). Given the typically positive correlation between EFZ and TAZ, we estimated EFZ_{PR} for these childhood data adjusting for the difference between the length of the small intestine for the childhood age and that for adults (16) (Table 2). In contrast to

TABLE 1 Total fecal Zn, intestinal excretion of EFZ, and EFZ per kilogram body wt in Guatemalan school children by maize group¹

Variables	Maize intervention group			
	Combined, <i>n</i> = 53	<i>lpa</i> 1–1, <i>n</i> = 19	Wild type, <i>n</i> = 19	Local, <i>n</i> = 15
Total fecal Zn, mg/d	6.64 ± 2.46	6.87 ± 2.51	6.19 ± 2.74	6.90 ± 1.98
EFZ, mg/d	1.56 ± 0.69	1.77 ± 0.82	1.32 ± 0.57	1.61 ± 0.63
EFZ, $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	0.07 ± 0.03	0.08 ± 0.04	0.06 ± 0.03	0.07 ± 0.02

¹ Values are means \pm SD. Variables did not differ in maize groups.

TABLE 2 EFZ_{PR} in children from less-developed countries adjusted for length of small intestine vs. adjustment for body wt¹

Group	Reference	n	Age, y	Wt, kg	Intestinal Length, cm	EFZ _{PR} ² mg Zn/d	EFZ _{PR} ³ mg Zn·kg ⁻¹ ·d ⁻¹
Guatemalan children	(This study)	53	4–13	22.3	450	1.94	0.06
Malawian children	(13)	23	8	22.1	440	2.01	0.07
Chinese children	(14)	43	1.9	10.9	400	1.22	0.07
Adult men	(5)	–	19–35	76	600	2.6	0.03
Adult women	(5)	–	19–35	61	600	2.3	0.04

¹ EFZ_{PR}: estimated excretion of endogenous Zn in the feces when quantity of Zn absorbed matches estimated physiologic requirement for Zn.

² Adjusted for small intestinal length: EFZ_{PR} × length of adult small intestine/length of small intestine at mean age of study population (16).

³ Adjusted for kilogram body wt.

expressing these EFZ_{PR} data as a function of body wt, when data are adjusted for differences in length of the small intestine compared with that of adults, the childhood data are lower rather than higher than adult data for each group of children.

At this stage in our understanding, relating experimental data for EFZ_{PR} to length of small intestine serves primarily to illustrate that there are physiologically plausible means of comparing EFZ_{PR} data from different studies that are more realistic than body wt. The pancreas also has a major role in the secretion of endogenous Zn into the lumen of the small intestine. However, we have been unable to identify quantitative data on developmental changes in indices of exocrine pancreatic function and could not, therefore, examine age-related differences in EFZ_{PR} in relation to exocrine pancreatic function. Whereas this limitation gives reason for caution in concluding that developmental changes in the length of the small intestine alone provide a comprehensive means of relating childhood EFZ_{PR} data to adult data, the relationship observed does serve to illustrate that the EFZ_{PR} data for these childhood studies are not necessarily abnormally high. Though perhaps requiring confirmation from studies of the intestinal excretion of endogenous Zn in toddlers and school-aged children in apparently well-nourished children in North America and elsewhere, these results indicate the need for reexamination of the estimates of the National Institutes of Medicine (5). The latter estimates are clearly uncertain, being based on extrapolation from adult data and expressing estimates on a body wt basis for which there is no physiologic support. Alternatives to body wt adjustment require consideration.

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